# MODULATORS OF PIN1 ACTIVITY AND USES THEREOF

## RELATED APPLICATIONS

**[0001]** This application is a Continuation of PCT Patent Application No. PCT/IL2020/050043 having International filing date of Jan. 9, 2020, which claims the benefit of priority under 35 USC § 119(e) of U.S. Provisional Patent Application No. 62/790,133 filed on Jan. 9, 2019. The contents of the above applications are all incorporated by reference as if fully set forth herein in their entirety.

## SEQUENCE LISTING STATEMENT

**[0002]** The ASCII file, entitled 88213SequenceListing.txt, created on Jul. 9, 2021, comprising 2,487 bytes, submitted concurrently with the filing of this application is incorporated herein by reference.

## FIELD AND BACKGROUND OF THE INVENTION

**[0003]** The present invention, in some embodiments thereof, relates to pharmacology, and more particularly, but not exclusively, to newly designed compounds that covalently bind to, and/or modulate the activity of, Pin1 and to uses thereof, for example, in treating diseases associated with Pin1 activity.

**[0004]** Phosphorylation of Serine-Proline or Threonine-Proline motifs (pSer/Thr-Pro) by proline-directed kinases is a central signaling mechanism that is reported to be frequently deregulated in oncogenic pathways, driving cell transformation and downregulating apoptosis [Hanahan & Weinberg, *Cell* 2011, 144:646-674]. This motif can be isomerized (from cis to trans or trans to cis) by peptidyl-prolyl isomerase NIMA-interacting-1 (Pin1) [Lu and Zhou, *Nat Rev Mol Cell Biol* 2007, 8:904-916], which is the only phosphorylation-dependent isomerase amongst the approximately 30 peptidyl-prolyl cis-trans isomerases (PPIases) in the human proteome. This isomerization induces conformational changes that can impact substrate stability [Lam et al., *Mol Cancer* 2008, 7:91; Liao et al., *Oncogene* 2009, 28:2436-2445; Lee et al., *Nat Cell Biol* 2009, 11:97-105], activation [Chen et al., *Cell Death Dis* 2018, 9:883], subcellular localization [Ryo et al., *Nat Cell Biol* 2001, 3:793-801], and/or binding to interaction partners including Proline-directed kinases and phosphatases, which are mostly trans-specific [Xiang et al., *Nature* 2010, 467:729-733; Zhou et al., *Mol Cell* 2000, 6:873-883; Brown et al., *Nat Cell Biol* 1999, 1:438-443]. Pin1 is therefore an important mediator of proline-directed signaling networks, and frequently plays a role in cancer, of activating oncogenes and inactivating tumor suppressors [Chen et al., *Cell Death Dis* 2018, 9:883].

**[0005]** Several lines of evidence indicate that abnormal Pin1 activation is a key driver of oncogenesis.

**[0006]** Pin1 has been reported to be overexpressed and/or overactivated in at least 38 tumor types [Bao et al., *Am J Pathol* 2004, 164:1727-1737], by mechanisms which include transcriptional activation [Rustighi et al., *Nat Cell Biol* 2009, 11:133-142; Ryo et al., *Mol Cell Biol* 2002, 22:5281-5295] and post-translational modifications [Lee et al., *Mol Cell* 2011, 42:147-159; Rangasamy et al., *Proc Natl Acad Sci* 2012, 109:8149-8154; Chen et al., *Cancer Res* 2013, 73: 3951-3962; Eckerdt et al., *J Biol Chem* 2005, 280:36575-36583]. High expression is reported to correlate with poor clinical prognosis [Lu, *Cancer Cell* 2003, 4:175-180; Tan et al., *Cancer Biol Ther* 2010, 9:111-119], whereas polymorphisms that result in lower Pin1 expression is reported to reduce cancer risk [$L_1$ et al., *PLoS One* 2013, 8:e68148].

**[0007]** Pin1 has been reported to sustain proliferative signaling in cancer cells by upregulating over 50 oncogenes or growth-promoting factors [Chen et al., *Cell Death Dis* 2018, 9:883], including NF-κB [Ryo et al., *Mol Cell* 2003, 12:1413-1426], c-Myc [Farrell et al., *Mol Cell Biol* 2013, 33:2930-2949] and Notchl [Rustighi et al., *Nat Cell Biol* 2009, 11:133-142], while suppressing over 20 tumor suppressors or growth-inhibiting factors, such as FOXOs [Brenkman et al., *Cancer Res* 2008, 68:7597-7605], Bcl2 [Basu et al., *Neoplasia* 2002, 4:218-227] and RARa [Gianni et al., *Cancer Res* 2009, 69:1016-1026].

**[0008]** Furthermore, Pin1 depletion was reported to inhibit tumorigenesis in mouse models derived by mutated p53 [Girardini et al., *Cancer Cell* 2011, 20:79-91], activated HER2/RAS [Wulf et al., *EMBO J* 2004, 23:3397-3407], or constitutively expressed c-Myc [D'Artista et al., *Oncotarget* 2016, 7:21786-21798].

**[0009]** In addition, Pin1 inhibition has been reported to sensitize cancer cells to chemotherapeutics [Gianni et al., *Cancer Res* 2009, 69:1016-1026; Zheng et al., *Oncotarget* 2017, 8:29771-29784; Sajadimajd & Yazdanparast, Apoptosis 2017, 22:135-144; Ding et al., *Cancer Res* 2008, 68:6109-6117] and to radiation [Liu et al., *Nat Cell Biol* 2019, 21:203-213], and block the tumorigenesis of cancer stem cells [Rustighi et al., *Nat Cell Biol* 2009, 11:133-142; Ding et al., *Cancer Res* 2008, 68:6109-6117; Min et al., *Mol Cell* 2012, 46:771-783], which are involved in the development of drug resistance [Dean et al., *Nat Rev Cancer* 2005, 5:275-284].

**[0010]** Hennig et al. [*Biochemistry* 1998, 37:5952-5960] describes irreversible inhibition of several PPIases by juglone (5-hydroxy-1,4-naphthalenedione).

**[0011]** Kim et al. [*Mol Cancer Ther* 2009, 8:2163-2171] reports that inhibition of Pin1—e.g., by juglone—reduces angiogenesis associated with growth factor release by tamoxifen-resistant breast cancer.

**[0012]** Campaner et al. [*Nat Commun* 2017, 8:15772] reports that KPT-6566, a derivative of juglone, exhibits anti-cancer activity mediated by covalent inhibition of Pin1 and release of a quinone-mimicking drug that generates reactive oxygen species and DNA damage.

**[0013]** Wei et al. [*Nat Med* 2015, 21:457-466] reports that the anticancer activity of all-trans retinoic acid (ATRA) is mediated by inhibition of Pin1.

**[0014]** Kozono et al. [*Nat Commun* 2018, 9:3069] reports that the anti-cancer activity of the combination of arsenic trioxide and ATRA is mediated by noncovalent binding of arsenic trioxide to Pin1 and by enhancement by ATRA of arsenic trioxide cellular uptake, as well as by inhibition of Pin1 by ATRA.

**[0015]** However, Pin1's potential as drug target remains elusive because available Pin1 inhibitors lack the specificity and/or cell permeability to interrogate its pharmacological function in vivo [Lu & Hunter, *Cell Res* 2014, 24:1033-1049; Moore & Potter, *Bioorganic Med Chem Lett* 2013, 23:4283-4291; Fila et al., *J Biol Chem* 2008, 283:21714-21724].

**[0016]** Additional background art includes Blume-Jensen & Hunter [*Nature* 2001, 411:355-365]; Cheng et al. [*J Med*